# INHERITANCE IN THE ASEXUAL REPRODUCTION OF HYDRA

K. S. LASHLEY

A Dissertation Submitted to the Board of University Studies
of the Johns Hopkins University in Conformity
with the Requirements for the Degree of
Doctor of Philosophy

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# INHERITANCE IN THE ASEXUAL REPRODUCTION OF HYDRA

K. S. LASHLEY

*From the Zoölogical Laboratory of the Johns Hopkins University*

TEN FIGURES

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## I. INTRODUCTION

The theoretical bearing of the work of Johannsen upon pure lines was not widely appreciated by students of heredity for the first few years after its publication; the general applicability of Mendel's law had not been shown and, hence, the genotype conception was without the support since gained from the evi-

dence bearing upon the stability of the unit character. The first attempt to test the general application of Johannsen's conclusions was made by Elise Hanel. In 1908 she published the results of two years of experimental study of inheritance in the asexual reproduction of the fresh-water polyp, Hydra grisea, and drew conclusions which were quite in accord with the results obtained with self-fertilized lines of beans. Her results were soon called in question, however, by the work of Hase ('09) and the criticisms of Pearson ('10), who obtained such conflicting results that a re-investigation of the subject became necessary. The results of such a renewed, independent study of variation and inheritance in Hydra are presented in the present paper.[1] In order to make clear the questions at issue and the bearing of the results presented here upon these and the general problems of genetics, it will be necessary to review briefly the work of Hanel and the criticisms raised against it, especially as the latter are complicated and not clearly valid.

Before this is undertaken, however, some explanation of the terminology of the present paper may not be out of place in view of the past confusion in genetic literature. Johannsen ('13), following Shull, has employed the word 'clone' to describe a family descended from a single individual by asexual reproduction. The name 'biotype' applies to any group of organisms which show the same hereditary constitution ('genotype'). In the statistical terminology the 'distribution' is the natural arrangement of individuals showing variation ('variates') in classes. The 'mean' is the arithmetical average of the variates. The 'standard deviation' ($\sigma$) is an expression of the average extent of variation from the mean shown by the variates. The 'coefficient of variation' expresses the average percentage of variation from the mean in terms of the mean as unity. The 'coefficient of correlation' ($r$) represents an arbitrary measurement of the average degree of resemblance between correlated members of two series of variates. The 'coefficient of regression' ($R$) expresses the average extent to which the variations of one series follow the variations of the correlated series.

The nomenclature of the genus Hydra has been subject to frequent changes. The names which have been used most frequently by ex-

[1] This was undertaken at the suggestion of Prof. H. S. Jennings, to whom I wish to express my indebtedness for his assistance and kindly criticism during the course of my work. I wish also to thank Prof. B. E. Livingston for the use of the facilities of the Laboratory of Plant Physiology of the Johns Hopkins University during the summer of 1912.

perimental zoölogists are those of Linnaeus. Brauer ('08) applied the rule of priority, thus requiring the nomenclature of Pallas. In 1912 Bedôt criticised Brauer's revision and showed that the species, H. viridis L. should be retained. Since the experimental literature has employed the Linnaean nomenclature this has been used here in order to avoid the constant repetition of synonyms. The names employed are given below at the left, with the revision of Bedôt at the right.

| | |
|---|---|
| H. viridis L. | H. viridis L. |
| H. grisea L. | H. vulgaris Pall. |
| H. fusca L. | H. oligactis Pall. |
| | H. polypus A. Brauer. |

*Experiments of Hanel*

Hanel began her work in 1906 and during the two years of her experiments bred clones from 26 wild Hydras, obtaining records of nearly 7000 buds, the descendants, by asexual reproduction, of the original 26. Upon comparing these 26 'stem parents' with their immediate progeny she found that those with a large number of tentacles produced buds having, on the average, a greater number of tentacles than the corresponding buds of parents with few tentacles. From the progeny of the 'stem parents' taken singly Hanel selected polyps with a high, an intermediate, and a low number of tentacles and continued this selection for from two to seven generations. Averaging the results obtained, she found that within the single clone the mean number of tentacles of the progeny of individuals selected for four generations was slightly less in the group selected for a large number than in the group selected for a small number, whence she concluded that variations within the clone are not inherited.

*Criticisms of Hanel's results*

In 1909 Hase, at the suggestion of Plate, studied the relation of the number of tentacles of Hydra to the age of the polyp and to the number of tentacles of the buds. He verified the observations of Parke ('00) and Hanel that the number of tentacles increases with the age of the polyps, finding an average increase of 2.1 tentacles in a number of polyps kept under observation for 90 days. In recording her data Hanel had taken this fact

into consideration but, believing from observations upon mass cultures that the adult number of tentacles is proportional to the number which the polyps bear when they produce their first buds, she thought herself justified in computing the adult number of tentacles for young poylps which had produced a single bud. Hase concluded that the increase is not regular and that there is no true adult number of tentacles for any polyp.

Hase further believed that the number of tentacles which the buds have at the time of separation from their parents is not proportional to the number of tentacles which the parents bear at that time. He gave data to show that while the number of tentacles of the parents is increasing with age, that of the successive offspring of these parents remains constant and does not follow the increase shown by the parents. He also cut a number of polyps into two or three pieces and found that they did not regenerate the same number of tentacles that they had borne originally. From this he concluded:

> Die verschiedene Tentakelzahl ist eine reine Somation und besitzt keinerlei Erblichkeitswert. Als eine reine Linie (im Sinne von Johannsen) kann man daher die direkten ungeschlechtlichen Nachkommen einer Hydra nicht bezeichnen. Es ist daher auch nicht möglich charakteristische Typen zu isolieren und sie erblich konstant zu erhalten. Aus gleichem Grund kann man auch keine reinen Linien (im vorigen Sinne) aus einer Hydrapopulation durch Selektion sortieren.

This conclusion is far from justified, however, by the evidence which Hase presents. Records of two parent Hydras are given, which increased slowly in their number of tentacles from five to nine, together with records of all the progeny of each. If the two groups are averaged, as has been done in table 1, it is evident that the buds produced by these two parents after they had acquired a large number of tentacles have a higher average than those produced when the parents had few tentacles. Yet from these data, Hase concludes, "Wir sehen, zuerst, ein scheinbares Mitgehen der Tentakelzahl der Knospen mit der Mutter, aber bald tritt ein *völliger* Rückschlag ein." As I shall show later, this is not true for H. viridis, as it is probably not for H. fusca: the regression is only partial.

The experiment upon regeneration likewise fails to support the conclusion which Hase draws from it, since it shows only that the number of tentacles is not regenerated at its full value but fails to disprove that the number regenerated is not proportional to the original number. I have reviewed the experiments of Hase in some detail because they have been generally quoted as proving that the number of tentacles of Hydra is not an hereditary character, whereas, at best, they but serve to cast some doubt upon Hanel's conclusions.

TABLE 1

*Increase in the average number of tentacles of buds produced while their parents bore the numbers increasing from 5 to 9 (modified from Hase)*

| NUMBER OF TENTACLES BORNE BY PARENTS | MEAN NUMBER OF TENTACLES OF BUDS PRODUCED WHILE THE PARENTS BORE THE NUMBER OPPOSITE |
|---|---|
| 5 | 5.60 |
| 6 | 5.63 |
| 7 | 6.33 |
| 8 | 5.80 |
| 9 | 6.32 |

A much more serious criticism of Hanel's work, and of 'pure line' work in general, was made by Pearson during the following year. He subjected Hanel's data to a more thorough analysis than she herself had done and held that they by no means justified the conclusions drawn. Concerning the evidence for the existence of strains, diverse with respect to tentacle number, he says:

Hanel begins with a very careful investigation of the growth and environmental changes in the character selected, the number of tentacles of Hydra grisea. There is a general agreement with Parke's results that the number of tentacles changes with age, size, food and place of culture. Differences in these factors can produce very considerable differences in individuals and differences in the averages of differentially treated groups which can amount to as much as 0.5 to 0.8 of a tentacle. These are precisely the order of the average hereditary differences. Thus:

| Parent | | Offspring | |
|---|---|---|---|
| *No. of individuals* | *No. of tentacles* | *No. of individuals* | *No. of tentacles* |
| 9 | 6 | 364 | 6.943 |
| 9 | 7 | 310 | 7.296 |
| 4 | 8 | 166 | 7.344 |
| 4 | 9 | 125 | 7.383 |

It will be at once recognized that the differences here are rather less than many of the environmental differences and that there is no security that these 26 foundation Hydras are really represented by differentiated hereditary numbers of tentacles. Yet this table, as it stands, embraces Hanel's proof that the number of tentacles is an hereditary character in the 'pure line.' What evidence is there that any one of the numbers of tentacles attached to those 26 parents is really constitutional and not environmental?

When we consider that Hanel's experiments extended over a period of two years, that the different 'stem parents' were collected and bred at different seasons, and that no data are given by which it is possible to judge which of the races were kept under a uniform environment this criticism greatly reduces the value of her evidence for the existence of diverse genotypes. The second point of Pearson's criticism deals with the inheritance of the number of tentacles within the clone. The data were subjected to statistical analysis by Miss Elderton and the correlation between relatives was determined for different degrees of relationship, within the population composed of the 26 clones. A comparison of the correlations showed that there is a greater resemblance between siblings and between parents and their offspring than there is between 'uncle and nephew' or between grandparents and grandchildren. This, as Pearson points out, can not be due merely to the inclusion of diverse races in the tables of correlation but indicates that there is an inheritance of variations within the clone. From this evidence Pearson concludes that Hanel's records show, not what she believed them to show, but just the reverse; that regression is only partial, both in the population and in the clone; that variatons are inherited equally in both population and clone.

### *Analysis of Hanel's data*

Since this method of treating the data does not deal with individual clones it seemed advisable to carry the analysis somewhat farther. I have computed the mean number of tentacles of all individuals in each of Hanel's clones and the correlation between parent and offspring for each.[2] These are given in tables 2 and 3. The mean numbers of tentacles of the clones

TABLE 2

*The mean number of tentacles of Hanel's clones arranged in the order of magnitude*

| CLONE NO. | MEAN NO. OF TENTACLES | $\sigma$ | NO. OF TENTACLES OF STEM PARENT |
|---|---|---|---|
| 14 | 6.428±0.040 | 0.771 | 7 |
| 13 | 6.438±0.020 | 0.475 | 7 |
| 24 | 6.670±0.058 | 0.826 | 6 |
| 8 | 6.677±0.049 | 0.816 | 7 |
| 5 | 6.805±0.029 | 0.734 | 6 |
| 2a | 6.925±0.054 | 0.692 | 6 |
| 1 | 6.926±0.041 | 0.750 | 6 |
| 11 | 7.010±0.031 | 0.786 | 6 |
| 12 | 7.026±0.024 | 0.780 | 6 |
| 20 | 7.170±0.031 | 0.721 | 7 |
| 2b | 7.184±0.032 | 0.658 | 5 |
| 10 | 7.230±0.034 | 0.798 | 8 |
| 7 | 7.240±0.023 | 0.906 | 7 |
| 26 | 7.264±0.041 | 0.902 | 9 |
| 16 | 7.287±0.056 | 0.962 | 12 |
| 19 | 7.319±0.032 | 0.784 | 5 |
| 18 | 7.325±0.029 | 0.786 | 7 |
| 23 | 7.361±0.050 | 0.850 | 5 |
| 9 | 7.367±0.046 | 1.005 | 8 |
| 6 | 7.384±0.025 | 0.914 | 9 |
| 3 | 7.393±0.023 | 0.763 | 8 |
| 15 | 7.400±0.040 | 0 938 | 8 |
| 22 | 7.430±0.043 | 0.934 | 7 |
| 17 | 7.456±0.049 | 1.197 | 10 |
| 4 | 7.500±0.039 | 0.862 | 7 |
| 21 | 7.641±0.047 | 0.835 | 6 |
| 25 | 7.712±0.044 | 0.942 | 7 |

[2] Hanel records the variations in her clones in percentages; the tables contain many errors, the sum of the percentages included in one array ranging from 85 to 140 per cent. This leads to error in the means and correlations computed from the tables but these probably average out in the numerous constants determined.

form an almost unbroken series from the smallest to the largest, and, lacking evidence upon the environmental conditions, give little indication of the existence of diverse races. Further analysis indicates that the clones tended to become more alike after they had been kept under cultivation for some time. The average number of tentacles of the offspring of each of the 26 'stem parents' and the average of all their later descendants were computed and are given in table 4. The descendants of parents with extreme variations tend to revert to the mean.

The coefficients of correlation between parent and offspring within the clones (table 3) are extremely variable, but the

TABLE 3

*Correlation between parent and progeny within each of Hanel's clones*

| CLONE NO. | CORRELATION | CLONE NO. | CORRELATION |
|---|---|---|---|
| 1 | 0.170±0.054 | 14 | 0.057±0.050 |
| 2a | 0.336±0.067 | 15 | 0.323±0.040 |
| 2b | 0.469±0.040 | 16 | 0.050±0.066 |
| 3 | 0.010±0.029 | 17 | −0.073±0.040 |
| 4 | −0.009±0.040 | 18 | −0.060±0.037 |
| 5 | 0.234±0.037 | 19 | 0.072±0.041 |
| 6 | 0.142±0.026 | 20 | −0.087±0.041 |
| 7 | 0.036±0.028 | 21 | 0.708±0.030 |
| 8 | 0.068±0.066 | 22 | 0.007±0.047 |
| 9 | 0.006±0.047 | 23 | 0.178±0.059 |
| 10 | 0.009±0.042 | 24 | 0.000±0.067 |
| 11 | 0.040±0.037 | 25 | 0.128±0.046 |
| 12 | 0.031±0.030 | 26 | 0.104±0.047 |
| 13 | 0.048±0.040 | Mean of all | 0.101 |

TABLE 4

*Regression in the first and later generations of the descendants of Hanel's 26 'stem parents'*

| "STEM PARENTS"; NO. OF TENTACLES | FIRST GENERATION; MEAN NO. OF TENTACLES | ALL DESCENDANTS; MEAN NO. OF TENTACLES |
|---|---|---|
| 6 | 6.943 | 7.086 |
| 7 | 7.296 | 7.102 |
| 8 | 7.344 | 7.347 |
| 9 | 7.383 | 7.347 |

majority (75 per cent) are positive and the evidence favors the belief in an inheritance of variations within the clone. The great irregularity of the results, however, makes it impossible to draw from them any law of inheritance within the clone or even to be sure that there is any constant tendency toward the inheritance of variations.

### *Unsettled questions*

Thus the evidence from previous work bearing upon heredity in Hydra must be looked upon as inclusive and the chief problems of heredity and variation as still unsettled. Hanel maintains the existence of diverse races of Hydra which are distinct in their hereditary constitution. This is denied by both Hase and Pearson but upon different grounds. Hanel holds that, within a population, variations are inherited and that selection is effective in isolating the diverse races, within which there is no inheritance of variations. Hase denies that variations in the character studied are inherited at all and asserts that such variations are purely somatic. Pearson attempts to show that the variations are inherited both within the population and the clone. Analysis of Hanel's data shows that there is usually a correlation between parent and progeny within the clone; that this may be very high in some cases, and in others, negative, varying around an average of 0.101. Experimental analysis of the cause of this correlation is lacking.

The chief questions demanding further investigation seem to be the following: Will such a variable character as the number of tentacles of Hydra lend itself to genetic study? Are there other characters of Hydra which vary less within the individual? Given a character which is comparable in different individuals, do races, hereditarily diverse with respect to this character, exist? If there are such races, in what characters do they differ and are they numerous or few? Is there inheritance of individual differences within the population, and if so, what part do diverse races play in this inheritance? Is there actually a correlation between parent and progeny within the clone?

If so, how is it produced and what is its significance? Is there an inheritance of individual differences within the clone? What is the origin of the diversities between races?

## II. VARIATION IN HYDRA

The only variable character in Hydra considered in detail by previous workers has been the number of tentacles. At the beginning of the present experiments I examined a number of polyps in the hope of finding other variable characters suitable for genetic study. Size, body-form, color, relative length of the tentacles, reaction to mechanical stimuli, distribution of the nettle cells, diameter of the mature nematocysts, and reaction to light were compared. Of these only size seemed at all suitable for statistical study. Variations in practically all the characters of Hydra appear, not only between different individuals, but in the same individual at different times. Hase has shown that the number of tentacles, alone, varies too much in the individual to form a character suitable for statistical treatment and Plate ('13) has stated the necessity for the use of the number of tentacles at some definite stage in the development of the polyps in future studies of heredity. The same individual variability appears in the size of the polpys so that the size at some definite stage of development must be used in comparative studies.

The time when the bud separates from the parent marks the occurrence of certain definite physiological processes in the bud and, if the number of tentacles of the bud is taken at this time, it serves as an index of the state of development of the bud at the time when these processes are initiated. This number may be compared, in tests for heredity, either with the number borne by the parent at the corresponding age, or with the number which it bears when the given bud is produced. It is more difficult to find a stage where the size of different polyps is comparable. Growth is very rapid for the first few days after the buds are released and it is not always possible to take measurements of the new buds, as such measurements require a great deal of time. After four or five days for H. viridis, when the buds have matured

and begun to produce buds in their turn, growth is much slower, although the polyps continue to increase in size until the fifteenth day or later. For comparisons of parent and offspring, I have used measurements of the size at the age of seven days, or as near this as was practicable. The figures so obtained are greatly subject to chance variation but are problaby as dependable as any which can be obtained for so plastic an organism.

### *Variation in the number of tentacles of Hydra viridis*

The most frequent number of tentacles (the modal number) found in wild populations of H. viridis is usually 6, sometimes 7, and the normal range seems to be from 4 to 9. Table 5 shows, in percentages, the distribution of variations in H. viridis found by previous investigators. Both local and seasonal differences probably play a part in the production of the differences in these populations. The data shown in table 6 have been obtained

TABLE 5

*Distribution, in percentages, of variations previously recorded for Hydra viridis*

| NO. OF TENTACLES | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|
| Rand ('99) | | 2.5 | 42.0 | 40.0 | 12.0 | 2.0 | | | | |
| Hathaway ('99) | | 11.5 | 50.0 | 35.0 | 3.0 | | | | | |
| Parke ('00) I | | 32.3 | 48.4 | 12.9 | 4.8 | 1.6 | | | | |
| II | | 4.8 | 30.6 | 44.5 | 16.4 | 2.9 | 0.6 | | | |
| III | | 22.8 | 46.5 | 20.9 | 7.4 | 1.4 | 0.9 | | | |
| Reese ('09) | ? | ? | ? | 24.0 | 54.0 | 15.0 | ? | ? | ? | |
| Hase ('09) | 2.0 | 4.0 | 7.0 | 28.0 | 27.0 | 25.0 | 7.0 | 1.0 | 0.5 | 0.5 |

TABLE 6

*Distribution, in percentages, of variations in a population of Hydra viridis from Baltimore*

| | NO. OF TENTACLES | | | | | | | | MEAN NO. OF TENTACLES | $\sigma$ | NO. OF POLYPS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | |
| Oct., 1911. . | | 0.6 | 0.6 | 25.0 | 51.5 | 18.0 | 3.7 | 0.6 | $6.988 \pm 0.044$ | 0.8404 | 167 |
| Oct., 1913. . | 0.1 | 0.4 | 5.5 | 30.9 | 50.9 | 11.3 | 0.9 | | $6.696 \pm 0.015$ | 0.7332 | 1000 |
| Apr., 1914. . | | 0.7 | 0.7 | 11.4 | 41.4 | 37.9 | 7.1 | 0.7 | $7.393 \pm 0.050$ | 0.8827 | 140 |

from a population in a very small stagnant pond in the neighborhood of Baltimore. Very extensive seasonal changes are evident from the mean of the population at different times.

Numbers of tentacles less than 5 or greater than 9 probably can not be considered normal for H. viridis from this neighborhood. Buds formed with less than four tentacles are usually small and do not extend their tentacles. They either produce more tentacles quickly, or die. The only individuals with ten tentacles which I have found were small-bodied, dark in color, and produced buds very slowly. In H. grisea a large number of tentacles seems to present a rather unstable condition. Individuals with more than eight tentacles in my cultures have shown a tendency to divide lengthwise and thus reduce the number of tentacles to the mode. I have observed longitudinal division in only one specimen of H. viridis, which bore six tentacles at the beginning of division and gave rise to two polyps, each with six tentacles.

As individual polyps grow older they form additional tentacles, a condition first observed by Marshall ('82) in H. viridis. Hase has demonstrated a like condition in H. fusca and Hanel in H. grisea. Hanel has found that the addition of tentacles occurs even in polyps which are starving, although hunger tends to inhibit the process, as is clear from table 7. Parke found that the number of tentacles might be reduced and Hanel's table shows that this reduction is favored by starvation. My own

TABLE 7

*Changes in the number of tentacles of 129 specimens of Hydra grisea during one month's cultivation; after Hanel*

| | WITH FOOD | WITHOUT FOOD |
|---|---|---|
| Number of tentacles added | 45 | 19 |
| Number of polyps showing change | 29 | 14 |
| Number of tentacles absorbed | 2 | 8 |
| Number of polyps showing change | 2 | 6 |
| Number of polyps unchanged | 31 | 47 |
| Total number of polyps | 62 | 67 |
| Mean increase per individual | 0.69 | 0.16 |

data verify these results for H. viridis. Figure 1 shows the mean number of tentacles of about 30 polyps from two different clones at successive intervals of two days.

Besides the specific tendency to change the number of tentacles with advancing age, there are environmental factors which may produce variation in the number of tentacles of Hydra. Hanel's data, table 7, show that starvation partially inhibits

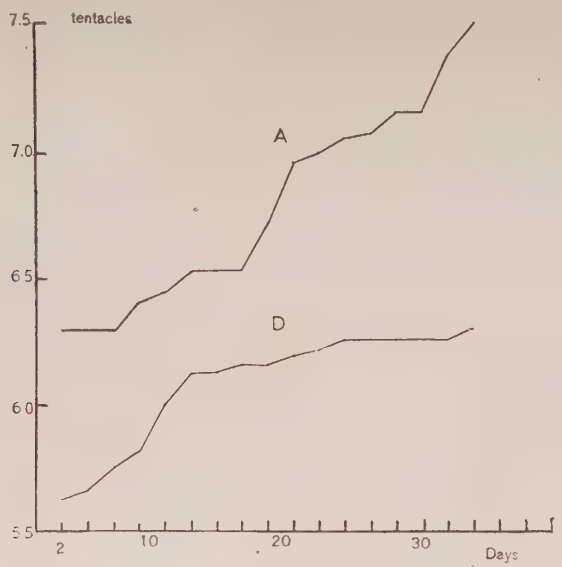


Fig. 1 Increase with advancing age in the average number of tentacles of polyps from two clones, *A* and *D*. The averages are based upon about 30 polyps from each clone. Clone *A* shows an average daily increase of 3.6 per cent; clone *D* an increase of 2.0 per cent.

the addition of tentacles and causes some to be absorbed. Parke has shown that other unfavorable conditions, such as stagnant water, have a like effect. Injury and regeneration of the oral end also leads to a reduction in the number of tentacles (Rand '99). These factors produce variation in adult Hydras, but further, as will be shown, they have a like effect upon the buds produced by these adults.

*Conditions producing like variations in parents and offspring*

*Age.* Corresponding to the increase in the number of tentacles with the age of the parents, the buds produced by old polyps have, on the average, more tentacles than those produced by young ones, that is, there is an increase in the number of

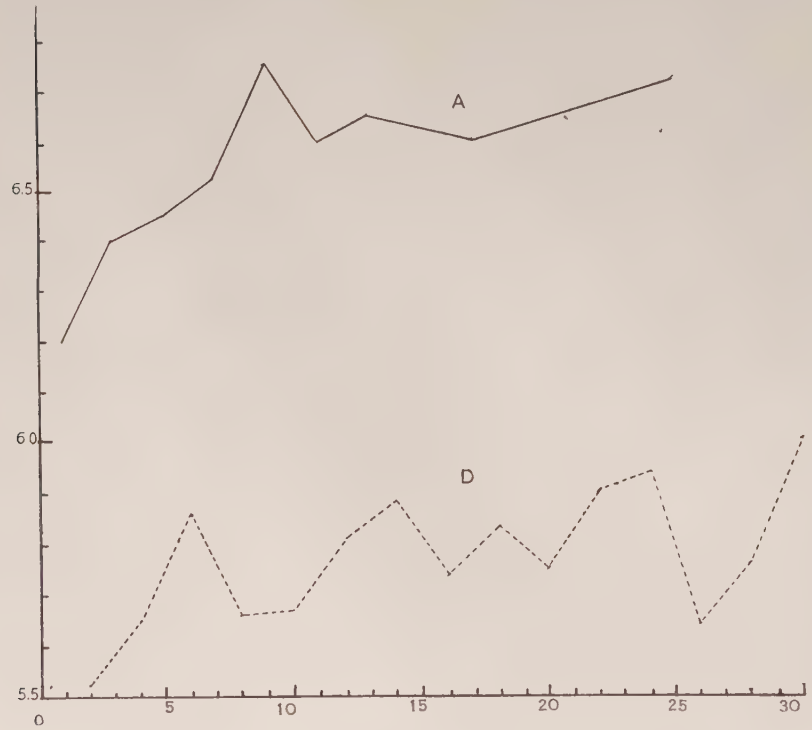


Fig. 2 Increase in the average number of tentacles of successive buds produced by the same parents. The ordinates represent the mean number of tentacles of the successive buds. The abscissae represent for Clone *A* the successive buds produced by 70 parents, for Clone *D* the buds produced on successive days after the 40 parents which furnished the basis of the curve began to produce buds.

tentacles of successive buds with the increasing age of the parent. Figure 2 shows this condition clearly. Data from two clones are included in this figure; for clone *A* the average number of tentacles of the first, third, fifth, seventh, etc., buds are given in the graph; for clone *D* the averages of all buds produced during suc-

cessive intervals of two days furnish the basis of the graph. The general trend of both lines is upward, although more markedly so in *A* than in *D*. The same relation for the two clones was found for the increase in the number of tentacles of the parents (fig. 1). The increase in the parental number of tentacles with age is due to some internal factor. Its relation to the increase in the number of tentacles of successive buds will be considered later. Besides this internal factor, there are various environmental agents which modify the number of tentacles of parent and bud in the same direction, and which must be taken into account in any study of inheritance.

*Effects of starvation.* One hundred polyps from a mass culture of a single clone (clone *A*) were divided at random into two groups of 50 each. One group was fed every second day with small crustacea, the other every sixth day. Both were placed in clean dishes of fresh water every second day, and each polyp was kept in a separate dish. The result of this method was that one group received about three times as much food as the other. The first result of the partial starvation of the six-day group was a reduction in the rate of budding. The well fed group produced an average of 0.203 buds per day for each polyp; the starved group produced only 0.074 buds per day, which gives a ratio of almost three to one, corresponding to the amount of food given. There was also a difference in the average number of tentacles of the buds produced by the two groups, the offspring of well fed parents having a slightly higher average than the others. This is shown in table 8. The difference here is small and may easily have been due to chance. The experiment was

TABLE 8

*Effects of starvation of parents upon the mean number of tentacles of their buds; all buds recorded within 24 hours after their release*

| | PARENTS | | BUDS | |
|---|---|---|---|---|
| | Mean no. of tentacles | No. of polyps | Mean no. of tentacles | No. of polyps |
| Fed..... | 7.18±0.06 | 50 | 6.32±0.04 | 153 |
| Starved..... | 7.18±0.07 | 50 | 6.24±0.06 | 61 |

TABLE 9

*Effects of starvation of parents upon the number of tentacles of their buds; second experiment*

| | PARENTS | | | BUDS | | |
|---|---|---|---|---|---|---|
| | Mean no. of tentacles | No. of polyps | Tentacles added by all parents | Mean no. of tentacles | No. of polyps | Difference |
| Clone *A* | | | | | | |
| Fed | 6.84±0.05 | 25 | 0 | 6.59±0.03 | 127 | |
| Starved | 6.80±0.05 | 25 | 0 | 6.37±0.09 | 44 | 0.22±0.09 |
| Clone *D* | | | | | | |
| Fed | 5.80±0.06 | 25 | 1 | 5.76±0.03 | 195 | |
| Starved | 5.92±0.06 | 25 | 0 | 5.20±0.06 | 44 | 0.56±0.07 |

repeated with polyps from two clones and results were obtained which made it certain that starvation of the parent leads to a reduction of the number of tentacles of its progeny. The results of this experiment are shown in table 9.

The parents recorded in this experiment were old polyps taken from mass cultures. Their mean number of tentacles already exceeded the means of the clones from which they came, so that any considerable increase in their numbers of tentacles during the brief time of the experiment was not to be expected. Hanel's data, however, prove that starvation tends to inhibit the addition of tentacles in parents and this experiment shows that the buds of starved parents have fewer tentacles than those of normal ones. This condition, occurring among a large number of polyps subjected to unequal and fluctuating environmental conditions would undoubtedly lead to a slight degree of resemblance between parent and progeny, even if there is no true inheritance of parental variations.

*Effect of injury.* Twenty mature Hydras were cut in two transversely and kept until both halves had regenerated. Each half was kept in a separate dish and the buds which it produced after regeneration was complete were recorded. Altogether, 287 buds were obtained from the 40 freshly regenerated polyps. The mean number of tentacles of the clone from which the original 20 were derived was, at this time, 6.91±0.03. The mean

number of tentacles of the original 20, the oral ends, was $7.05 \pm 0.09$. The mean number regenerated by the aboral ends was $6.42 \pm 0.09$. The regenerated oral ends produced 146 buds, the aboral ends, 132 buds within 28 days after the operations. The mean number of tentacles of the buds from the oral ends was $6.88 \pm 0.03$; that of the buds from the aboral ends was $6.59 \pm 0.04$; the difference here is $0.29 \pm 0.004$ in favor of the offspring of the polyps which were required to carry out the lesser regenerative processes. Clearly, the same factors which cause a reduction in the number of tentacles in the parents (the call for an expenditure of energy in regeneration) cause also a reduction in the number of tentacles of their offspring.

In order to test the length of time during which the after-effects of injury to the parents influence the number of tentacles of the buds, the mean number of tentacles of all first, all second, etc., buds produced after regeneration by the oral and aboral ends was computed. This is shown in figure 3. After the ninth bud the graphs are based upon too small numbers to be significant. It is clear from this figure that immediately after regeneration the buds produced have few tentacles and that the number of tentacles of successive buds increases rapidly until, after five or six have been formed, the normal condition is regained.

These experiments show that certain environmental agents, acting upon Hydra, tend to produce variation in a given direction in both parent and offspring, either, as in the first case, by acting simultaneously upon both parent and bud, or, as in the second case, by affecting the bud only indirectly through a reduction in the vitality of the parent. Although only two such agents have been studied experimentally it is probable that many others have a like effect. Parke has shown that the bacterial conditions leading to depression in his cultures brought about a reduction in the number of tentacles of the polyps. I have found that polyps which have just recovered from depression tend to produce one or more very minute buds with few tentacles. Polyps kept in water of high salt content become small and dark-colored and produce small dark-colored buds.

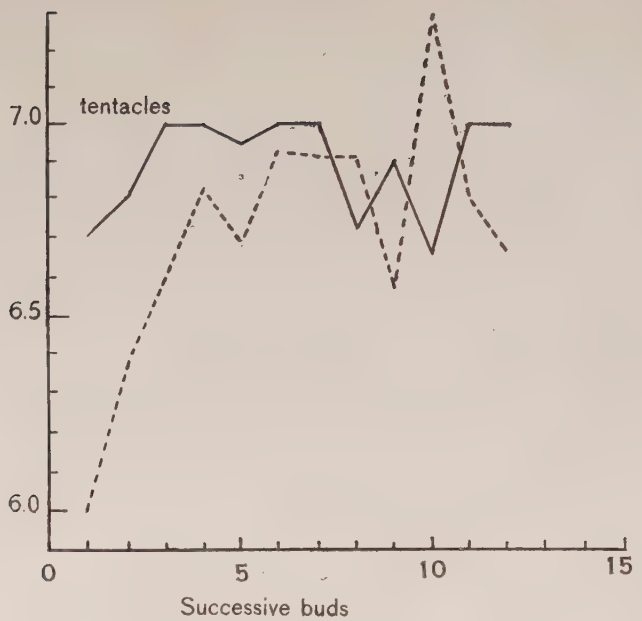


Fig. 3 The mean number of tentacles of the successive buds produced by polyps after regeneration; (——) buds produced by regenerated oral ends; (– – –) buds produced by regenerated aboral ends; all polyps were taken from the same clone.

Temperatures much above or below the optimum have an effect like that of high salt content. The results of the action of these agents resemble the cases of temporary parallel induction found in parthenogenetic reproduction (Agar '13) but the mechanism of the action is probably much simpler since we are dealing here only with a congenital reduction in vigor.

## III. EXISTENCE OF DIVERSE RACES

In addition to these variations resulting from environmental action, are there differences between the individuals of a population due to some internal, hereditary factors? This question has been tested by breeding a number of clones of H. viridis under similar cultural conditions.

*Experimental methods*

Hydra is easily cultivated in the laboratory, provided that a constant supply of food is available. The polyps used in the experiments were collected from various sources, usually from ponds containing Nitella or Elodea. In the greater part of the work each polyp was kept separately in a small Stender dish with about 5 cc. of water. It was found necessary to wash these dishes in boiling water every second day in order to keep down bacterial growths and maintain the polyps in good health. A small species of Cyclops was used exclusively as food. Attempts to breed them in sufficient numbers in the laboratory failed and all food material was obtained by towing with a plankton net in an artificial pond. The supply so obtained was further concentrated so that from one to two hundred Cyclops were given to each Hydra every second day. (A healthy specimen of H. viridis will eat ten or more Cyclops daily and the large number supplied allowed each polyp to capture as many as it could eat). At first, a growing stem of Elodea was kept in the culture with each Hydra but as this was found to be unnecessary and a possible source of contamination it was discontinued after the first experiment.

All the cultures were kept at approximately the same level upon a broad table-top where they were exposed to uniform conditions of light and temperature. The cultures of various clones were divided into small groups and the order in which these were fed and arranged upon the table was varied from day to day. While conditions may have varied in the different individual cultures, there seems to be no reason for believing that such differences did not average out in each of the clones, considered as a whole.

Besides the individual cultures a number of mass cultures of different clones were kept under similar conditions. Numbers of polyps were placed in 6-inch battery jars filled with water from the food pond. The water in the cultures was renewed weekly, and where two clones were being bred for comparison the water from the culture jars containing the two clones was interchanged frequently.

The number of tentacles of the polyps was counted under the Zeiss binocular, and in the case of the polyps in individual cultures the number of tentacles of the buds was recorded within 48 hours of the time when they were released from the parents. The numbers of tentacles of the parents were recorded also at the time when each bud was produced. Measurements of size were made in the following way: The polyps, contracted, were placed on a grooved slide under a compound microscope. When they expanded until the length was about four times the diameter, an outline of the body was made with a camera lucida. The area of this outline was measured with a planimeter and from this, treated as the area of the longitudinal section of an elipsoid, the volume was computed. This method is subject to about 25 per cent error, but is much more accurate than measurements of a single diameter. The constants given in the following pages were computed

by the simple statistical formulae devised by Pearson and others (Davenport '04; Yule '12). In the computation of the standard deviations for size Sheppard's correction was *not* used. All calculations were made with the aid of the 'Brunsviga' computing machine, and later repeated *de novo*.

*Detailed examination of two clones*

On June 10, 1912, twenty-five specimens of Hydra viridis, collected from a limited area in a small undrained pond, were isolated in individual culture dishes. They began to multiply rapidly under the favorable conditions of the cultures. The new-formed buds were each placed in a separate dish, labeled, and the number of their tentacles recorded within 48 hours after they separated from the parents. After a few days it was found impossible to care for all the descendants of the stem mothers and it seemed best to discard the majority of the clones. Two of them, *A* and *D*, which seemed somewhat diverse were retained. It soon became necessary again to reduce the number of cultures, as not more than 300 animals could be cared for at one time. An equal number of each of the two clones was therefore selected for high, intermediate, and low numbers of tentacles, and the cultures were so arranged as to give not less than ten progeny from as many parents as possible. Since the selections were approximately equal in both directions from the means of the clones, the average number of tentacles of the two may be used for comparison, although the coefficients of variation may be affected and complications are introduced in the analysis of the data for the problem of inheritance within the clone.

The breeding was continued until the latter part of August, when the first experiment was brought to a close, and the polyps on hand were placed in mass cultures and left for two weeks. At the end of this time a single polyp was taken from a mass culture of each of the clones and used as the starting point of a subordinate clone ($A'$ and $D'$) bred in individual cultures as in the first experiment, except that no Elodea was included in the cultures.

*Differences in number of tentacles and size.* The total number of pedigreed descendants of 'stem mother' *A* obtained before

September was 1353, of 'stem mother' *D*, 1395. The distribution of the variations in the number of tentacles of the two clones is given in table 10. The means and standard deviations computed from these figures are:

| | *Mean* | $\sigma$ | |
|---|---|---|---|
| Clone *A* | 6.463±0.013 | 0.7371 | Tentacles |
| Clone *D* | 5.739±0.011 | 0.6086 | Tentacles |
| Difference (*A* − *D*) | 0.724±0.017 | | Tentacles |

The difference between the average numbers of tentacles borne by polyps from the two clones is here many times its probable

TABLE 10

*The distribution of variations in the number of tentacles of the descendants of 'stem mothers' A and D*

| NUMBER OF TENTACLES | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|
| Number of polyps bearing each number of tentacles | | | | | | |
| Clone *A* | 9 | 93 | 590 | 589 | 69 | 3 |
| Clone *D* | 30 | 394 | 883 | 86 | 2 | 0 |
| Percentage of all polyps bearing each number | | | | | | |
| Clone *A* | 0.6 | 6.9 | 43.6 | 43.5 | 5.2 | 0.2 |
| Clone *D* | 2.2 | 28.2 | 63.3 | 6.2 | 0.1 | |

error and proves that the two clones differed either in their hereditary constitution or in the environment to which they were subjected. The conditions of the experiment seem to rule out the latter possibility and further evidence against an environmental cause of the difference was given by further experiments with the clones. The second part of the experiment, beginning with the establishment of the two subordinate clones *A′* and *D′*, from single individuals taken out of mass cultures gave 204 pedigreed descendants from stem mother *A′* and 153 descendants of stem mother *D′*. The number of tentacles of these polyps is shown in table 11. The constants for the distributions given are:

| | *Mean number of tentacles* | $\sigma$ |
|---|---|---|
| Clone $A'$ | $6.907 \pm 0.026$ | 0.557 |
| Clone $D'$ | $5.844 \pm 0.029$ | 0.537 |
| Difference $(A' - D')$ | $1.063 \pm 0.039$ | |

After two weeks in mass culture the difference between the two clones persisted and was, indeed, increased by 35 per cent. The significance of this increase will be discussed later.

TABLE 11

*Variations in clones $A'$ and $D'$*

| NUMBER OF TENTACLES | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|
| Number of polyps | | | | | |
| Clone $A'$ | | 3 | 33 | 148 | 20 |
| Clone $D'$ | 2 | 29 | 114 | 7 | 1 |

Similar results were obtained from populations of the two clones grown in mass culture. The results from five pairs of mass cultures will be considered.

Two battery jars were filled with pond water; to each a few Elodea stalks free from foreign Hydras were added; and each was inoculated with 50 polyps, the one from Clone $A$, the other from Clone $D$. Cyclops were added every week for three weeks. At the end of a month 100 polyps were taken from each culture and the numbers of their tentacles were recorded. The variations were distributed as shown in table 12, I. The constants obtained from this are:

| | *Mean number of tentacles* | $\sigma$ |
|---|---|---|
| Clone $A$ | $6.56 \pm 0.056$ | 0.8284 |
| Clone $D$ | $5.86 \pm 0.033$ | 0.4903 |
| Difference $(A - D)$ | $0.70 \pm 0.060$ | |

Five weeks later samples were again taken from these cultures. Their variations are shown in table 12, II. The constants for these groups are:

| | *Mean number of tentacles* | $\sigma$ |
|---|---|---|
| Clone $A$ | $6.797 \pm 0.042$ | 0.5038 |
| Clone $D$ | $5.457 \pm 0.045$ | 0.5539 |
| Difference $(A - D)$ | $1.340 \pm 0.061$ | |

TABLE 12

*Distribution of variations in samples taken from mass cultures*

| NUMBER OF TENTACLES | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|
| I After three weeks with plentiful food | | | | | |
| Clone *A* | 1 | 9 | 33 | 47 | 10 |
| Clone *D* | | 20 | 74 | 6 | |
| II After five weeks more under same conditions | | | | | |
| Clone *A* | | 1 | 13 | 48 | 2 |
| Clone *D* | 2 | 34 | 34 | | |
| III After one month with little food | | | | | |
| Clone *A* | | 1 | 17 | 28 | 4 |
| Clone *D* | | 26 | 24 | | |
| IV After three months in mass cultures | | | | | |
| Clone *A* | | 1 | 12 | 33 | 4 |
| Clone *D* | | 25 | 24 | 1 | |

Polyps from mass cultures to which no food had been added for one month showed the distribution of table 12, III. The constants for these groups are:

| | *Mean number of tentacles* | $\sigma$ |
|---|---|---|
| Clone *A* | $6.70 \pm 0.06$ | 0.6403 |
| Clone *D* | $5.48 \pm 0.05$ | 0.4995 |
| Difference $(A - D)$ | $1.22 \pm 0.08$ | |

The samples recorded in table 12, IV, were taken from mass cultures three months old. The constants for these groups are:

| | *Mean number of tentacles* | $\sigma$ |
|---|---|---|
| Clone *A* | $6.80 \pm 0.06$ | 0.6000 |
| Clone *D* | $5.52 \pm 0.05$ | 0.5380 |
| Difference $(A - D)$ | $1.28 \pm 0.08$ | |

The Hydras in one pair of cultures were given very little food for three months and the water in the cultures was changed very rarely. As a result of these unfavorable conditions the polyps in both cultures were very much reduced in size and in number of tentacles. Clone *A* was most affected but the difference between the clones persisted even here, as the following constants for these populations show:

| | *Mean number of tentacles* | $\sigma$ |
|---|---|---|
| Clone *A* | 5.519±0.035 | 0.5325 |
| Clone *D* | 5.346±0.033 | 0.4970 |
| Difference (*A* − *D*) | 0.173±0.048 | |

The second character studied in these clones was the size of the polyps when about one week old. The measurements include 184 individuals of Clone *A* and 182 of Clone *D*. The

TABLE 13

*Distribution of variations in the size of clones A and D*

| | CUBIC MILLIMETERS | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.00 | 0.16 | 0.32 | 0.48 | 0.64 | 0.80 | 0.96 | 1.12 | 1.28 | 1.44 | 1.60 | 1.76 | 1.92 |
| Clone *A* | | 10 | 25 | 22 | 28 | 28 | 26 | 13 | 11 | 8 | 4 | 1 | 8 |
| Clone *D* | 24 | 78 | 55 | 17 | 7 | 1 | | | | | | | |

distribution of the variations is shown in table 13. The mean sizes of the two clones are:

| | *Mean size cu. mm.* | $\sigma$ *cu. mm.* |
|---|---|---|
| Clone *A* | 0.869±0.021 | 0.4288 |
| Clone *D* | 0.322±0.022 | 0.1504 |
| Difference (*A* − *D*) | 0.547±0.023 | |

The mean age at the time of measurement for members of the two clones was:

Clone *A* 7.1 days.....Clone *D* 7.8 days

The difference in size is even more pronounced than the difference in the number of tentacles: individuals from Clone *A* were, on the average, more than twice as large as those from Clone *D*.[3] The difference in the appearance of the polyps is shown in figure 4. The numbers measured are relatively small but they extend

[3] An attempt was made to determine whether the difference in size was due to a difference in the size of the individual cells, in accordance with the theory of the relation between cell and body size advanced by Popoff ('08). Because of the great mobility of Hydra, accurate measurements could be obtained only for the mature nematocysts. In this character the two clones were identical and if it furnishes an index to the size of other cells the difference between the clones was due to a difference in the number and not the size of the body cells.

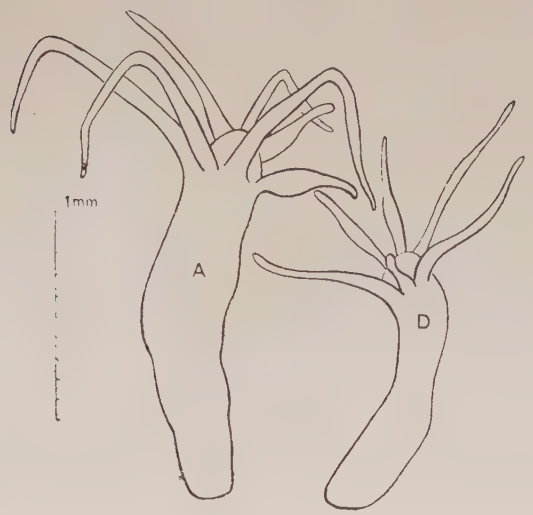


Fig. 4 Diagram of the size relations of Clones *A* and *D*. From camera drawings of polyps of mean size.

over more than half the period of cultivation so that environmental differences should have averaged out. The polyps measured were selected because of their relationships, so that the personal factor, an unconscious selection of extremes, can have played no part in producing the difference.

All the differences recorded are between groups which had been kept under environmental conditions as nearly uniform as possible. The polyps in individual cultures were fed in irregular order in order to insure a uniform distribution of food. In the mass cultures the parallel series of the two clones were given uniform treatment and an interchange of water in the parallel cultures was made frequently. The evidence seems to prove conclusively that some internal, hereditary factor caused the differences between the clones.

This conclusion is still further verified by an analysis of the data from the individual cultures. In order to test the constancy of the difference, the time during which the clones were cultivated was divided arbitrarily into intervals of five days and the mean number of tentacles of all buds produced by each clone during each five-day period was computed. Table 14 and figure

TABLE 14

*Constants for the number of tentacles of the buds produced by clones A and D during successive five-day intervals from June 25 to August 23*

| NO. OF PERIOD | CLONE *A* | | CLONE *D* | | (*A* − *D*) |
|---|---|---|---|---|---|
| | Mean no. of tentacles | $\sigma$ | Mean no. of tentacles | $\sigma$ | Tentacles |
| 1 | 6.530±0.099 | 0.6056 | 5.500±0.168 | 0.5000 | 1.030±0.194 |
| 2 | 6.115±0.118 | 0.8913 | 5.462±0.091 | 0.6919 | 0.653±0.149 |
| 3 | 6.223±0.046 | 0.7280 | 5.479±0.036 | 0.6204 | 0.744±0.053 |
| 4 | 6.142±0.055 | 0.8478 | 5.345±0.027 | 0.5679 | 0.796±0.061 |
| 5 | 6.233±0.055 | 0.7754 | 5.441±0.036 | 0.6257 | 0.792±0.065 |
| 6 | 6.552±0.031 | 0.6529 | 5.796±0.029 | 0.5532 | 0.756±0.042 |
| 7 | 6.795±0.030 | 0.6268 | 6.103±0.031 | 0.6386 | 0.692±0.043 |
| 8 | 6.681±0.033 | 0.6334 | 5.958±0.023 | 0.4542 | 0.723±0.040 |
| 9 | 6.439±0.036 | 0.6499 | 5.757±0.029 | 0.5078 | 0.682±0.046 |
| 10 | 6.236±0.038 | 0.7502 | 5.787±0.030 | 0.4980 | 0.449±0.042 |
| 11 | 6.396±0.043 | 0.5958 | 5.787±0.036 | 0.5376 | 0.972±0.056 |
| 12 | 6.384±0.090 | 0.6836 | 5.775±0.090 | 0.4875 | 0.609±0.128 |

5 show the result of this analysis. Throughout the entire experiment the difference between the clones persisted. By July 25 Clone *D* had increased in the average number of tentacles of its buds until it equaled the earlier average of Clone *A* (June 30) but this was accompanied by a corresponding increase in the average of Clone *A*. The figure shows that the difference remained fairly constant in spite of wide fluctuations in the absolute values of the means of the clones.

*Other differences between the clones.* There is a marked difference between the clones with respect to the age at which reproductive maturity is reached, the age at which the parents produce their first bud. This is shown in the following tabulation:

| | *Mean age of parents when first bud was produced* | $\sigma$ | *No. of parents* |
|---|---|---|---|
| Clone *A* | 4.806±0.103 days | 2.422 | 248 |
| Clone *D* | 3.748±0.074 days | 1.755 | 253 |
| Difference (*A* − *D*) | 1.058±0.127 days | | |

The rate of reproduction of the clones differed slightly, that of Clone *A* being somewhat greater than that of Clone *D*. The difference, based upon 33 parents of each clone which produced more than 10 buds is 0.0542 buds per day. This difference is

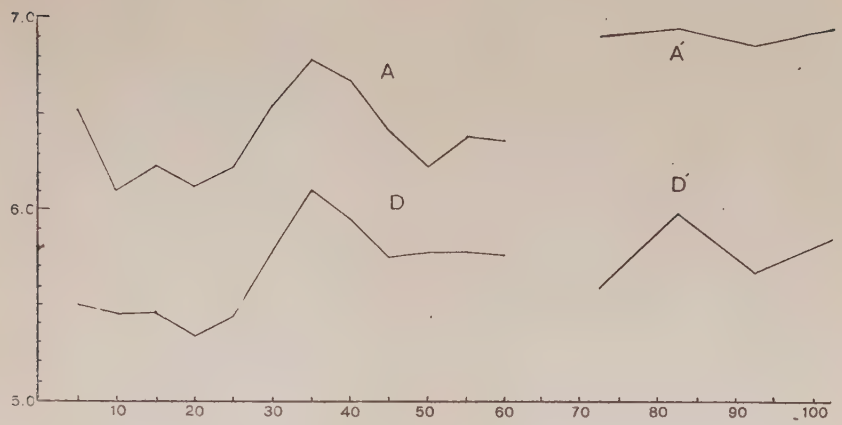


Fig. 5 The mean number of tentacles of the buds produced by Clones *A* and *D* during successive intervals of five days. The mean number of tentacles of Clones *A'* and *D'* are included at the right as these form a natural continuation of the earlier experiment.

not greater than its probable error and is probably not significant. The equality in reproductive rate is shown by the fact that while no attempt was made to obtain equal numbers in the two clones during the experiment, the numbers bred were, after two months, almost equal (1353 and 1395).

Other differences between the clones which can not be expressed quantitatively were apparent in the living animals. Clone *D* was, as a rule, somewhat darker in color than Clone *A* and somewhat more resistant to unfavorable conditions. Polyps of Clone *A* showed a greater tendency to cling to the surface film and were more active than those of Clone *D*.

*Nature of the difference between Clones A and D.* The foregoing data prove that the two clones studied are distinct through the presence of some internal, hereditary factor. The 'Reaktionsnormen' of the two are different. But whether this difference is genotypic in the sense of being the result of a fixed constitution of the germ-plasm or whether it is due to a spurious heredity like that by which the green color is transmitted is not clear. The agents, other than the hereditary constitution, which might produce such clonal diversities are the direct action of the

environment, the persistent effects of former diversities of environ ment, variations in the commensal relations of the polyps and their zoochlorellae, parasitic infection of one clone, and differences in clonal age from the fertilized egg. The first of these is eliminated by the methods of the experiment. It has been shown that the after effects of injury and 'depression' are only temporary. Experiments attempting to produce permanent modification by extremes of temperature, chemical agents, and artificially induced 'depressions' have been unsuccessful; diversities are readily produced but they persist for only a few days.

Possible variations in the commensal relations of H. viridis are more difficult to control. Microscopic examination of the two clones failed to reveal any difference in the green bodies of the two clones. As a test of the influence of commensalism upon variations I partially removed the zoochlorellae from some individuals of Clone *L* (see below) by the method devised by Whitney ('06–'08). During immersion in glycerin the polyps became smaller but when restored to normal conditions they ate regularly and resumed their original size before beginning to bud. The buds, practically white in color, did not seem otherwise different from the normal members of the clone. I never succeeded in getting out all the zoochlorellae from the polyps so that not enough buds for statistical study could be obtained before the polyps resumed their normal color.

Five parasites of Hydra have been reported: Amoeba hydroxena Entz, Balantidium hydrae Entz, a species of Ophryoglena (Entz '12), Trichodina pediculus Ehr. (Clarke '65), and Kerona polyporum Ehr. The true parasitic nature of only one of these, A. hydroxena, has been demonstrated. Trichodina did not occur in any of the pedigreed cultures recorded here, although it is common in wild populations. In October, 1913, an epidemic of A. hydroxena broke out in my cultures, destroying all of them. The symptoms of infection by this parasite are easily recognizable and there is no possibility that it occurred in the earlier cultures. Kerona occurs rarely in wild populations here but did not appear in my cultures; the other two parasites have

never been seen in this locality. The fungus mentioned by Rand ('99) is evidently not a parasite of Hydra.

Finally, there is no direct evidence bearing upon the question of the effects of the age of the clone upon its variations. It has been impossible to secure clones of known age since in this locality sexual reproduction in H. viridis seems to be almost completely suppressed. Hydras from wild populations have been examined at all seasons during the past three years and in this time not one with ovaries and only seven with spermaries have been found. The treatment by which Whitney ('07) induced sexual reproduction was tried but without success. These facts seem to justify the conclusion that sexual reproduction plays but little part in the life history of the green polyp.

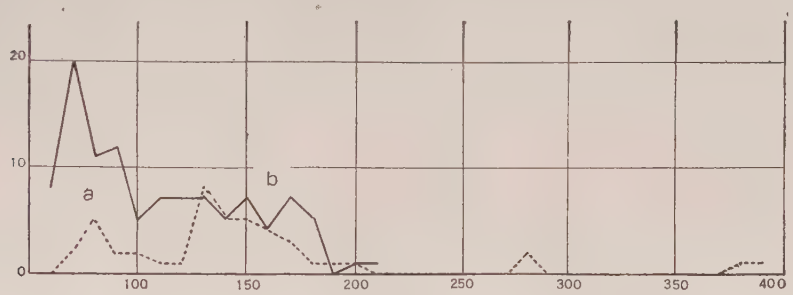


Fig. 6 The number of polyps (ordinates) which died at the ages in days recorded on the abscissae, during Hase's experiments. (——) H. fusca, (- - - -) H. grisea.

In the development of theories of 'life cycles' in micro-organisms the phenomena of 'depression' in Hydra have had a prominent place. R. Hertwig, in particular, has sought to emphasize the relations of 'depression' to sexual reproduction, but the work of Frischolz ('09) seems to prove that no such relation exists. Practically all studies of depression have been carried out with polyps in mass cultures and there is no evidence that the epidemics of depression reported were not due to environmental rather than to internal factors. The ease with which depression may be induced by unfavorable environment and cured by putting the polyps in clean water makes it practically certain that depression is a pathological condition.

The only other work bearing upon the question of age is that of Hase, who studied the age of individual polyps. He found a mean age of 55.2 days for H. fusca and 94.8 days for H. grisea, with the distribution

of ages at death shown in figure 6. The irregular form of the curves and the fact that the vast majority of the polyps died at a relatively early age scarcely speaks in favor of an 'Altersschwäche.' The curves are similar enough in form to suggest that the deaths were due to two periods of unfavorable conditions (*a* and *b*) in his cultures.

At no time have I observed an epidemic of depression in the individual cultures belonging to the same clone; less than 1 per cent of the polyps have shown depression and always the closest relatives of these remained normal, a condition which is not at all in harmony with theories of clonal senescense. Further, during the four months that the clones were kept under observation, including more than 20 asexual generations, there was no significant change in the character of either clone. The reproductive rate, the first character modified by a reduction of the vitality of the polyps, was as high at the end of the experiment as at the beginning. This furnishes some evidence against the belief that the diversities are due to the clonal age but it is not conclusive.[4]

*The existence of other races*

A comparison of the standard deviations of the Clones *A* and *D* reveals the fact that clone *A* was always the more variable of the two. This difference suggested that Clone *A* was not pure and an examination of other evidence quickly confirmed this view. The variations of the clone tended to a dimodality which does not appear in any other race studied and the greater variability and dimodal form of the variation curve did not persist in Clone *A'* which was descended from a single polyp taken from Clone *A*. The records of the clone were reviewed in order to test whether the greater variability was characteristic of the race or was the result of the inclusion of diverse types as is suggested by the condition in Clone *A'*. One subordinate clone represented by the direct line of descent A1A2b1a1a2a1a (the

[4] Agar ('14) has shown that diverse clones produced by parthenogenetic reproduction may be produced by different eggs hatched at the same time. The differences here are clearly not due to the ages of the clones. Lang ('92) found that only one germinal layer of Hydra takes part in the formation of the buds, and this work, while unconfirmed, suggests that the differences between parthenogenesis and budding may not be so great as is generally supposed.

successive buds of alternate generations being labled with the series of letters and numerals) showed an average number of tentacles considerably lower than that of the remaining portion of the clone. The polyp *A1A* was recorded as small, with abnormal tentacles, rapidly becoming normal. The mean number of tentacles of the fraternity from which it came was $6.809 \pm 0.062$, that of its immediate progeny was $6.200 \pm 0.023$, giving a reduction of $0.609 \pm 0.067$ tentacles in one generation.

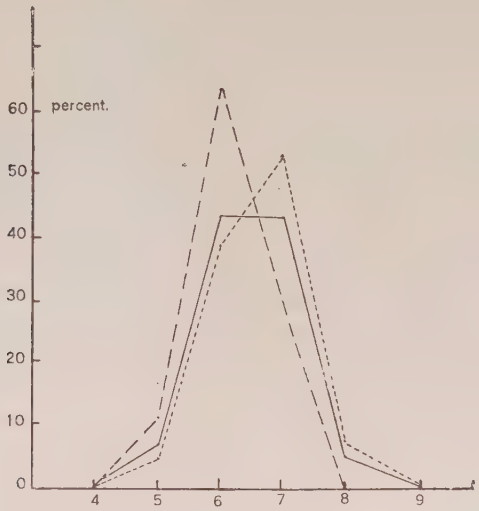


Fig. 7 The distribution of variations in the number of tentacles of Clone *A* (——) and of the subordinate clones *A1A* (— — —) and *A* − (*A1A*) (- - - -).

The mean of all the descendants of *A1A* (437) was $6.121 \pm 0.020$: that of the remainder of the clone was $6.624 \pm 0.013$ giving a difference of $0.503 \pm 0.024$. The distribution of variations in the two subordinate clones, separately and combined, is shown in figure 7. The dimodality of Clone *A* is clearly the result of the combining of these two groups (neither subordinate clone breaks up upon further analysis nor does any other method of dividing Clone *A* reduce its dimodality. The appearance of this diversity gives no evidence of a cumulative inheritance of variations. The change was quite sudden, resulting in a difference after one generation as great as the difference in any later gener-

ation. The individual *A1A* must be looked upon either as a mutant or as, more probably, a polyp of another clone introduced by accident into the culture.[5] It seems to be hereditarily different both from Clone *D* and from the remainder of Clone *A*.

Diverse races which can be recognized at a glance are extremely rare in the populations near Baltimore. Several times fifty

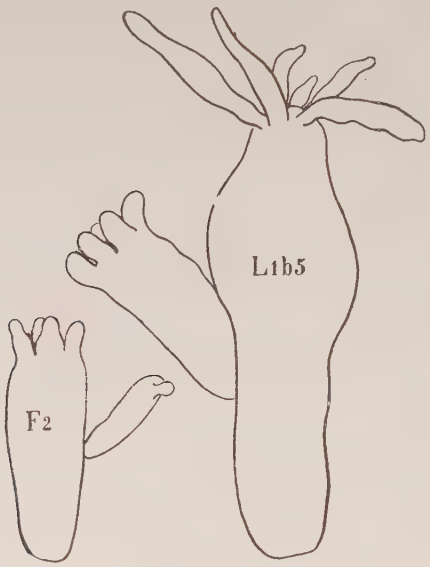


Fig. 8 Diagram of the size relations of two clones kept under similar conditions, *L* and *F*. From camera sketches of individuals of estimated average size.

or more clones have been started with polyps from different localities but only twice have distinct races appeared which were recognizable within a few generations. The first of these were Clones *A* and *D*. In August, 1913, a small polyp was found which produced very small buds. At first it seemed unhealthy but the buds increased in size and began to multiply rapidly. Unfortunately the culture could not be continued and the clone was

[5] The opportunity for contamination was given by the use of Elodea stalks in these cultures, as in the early part of the experiment no certain method of freeing them from foreign Hydras had been devised.

TABLE 15

*A comparison of the mean number of tentacles of clones T, L, and R*

| CLONE | NO. OF TENTACLES OF STEM MOTHER | MEAN NO. OF TENTACLES OF DESCENDANTS | NO. OF DESCENDANTS |
|---|---|---|---|
| T..... | 6 | 6.553±0.058 | 56 |
| L..... | 6 | 6.282±0.049 | 46 |
| R..... | 8 | 6.650±0.081 | 40 |
| T..... | | 6.513±0.033 | 154 |
| L..... | | 6.184±0.033 | 152 |

Difference ($T - L$) 0.328±0.046

lost before many individuals had been obtained. However, the difference between this and another clone, *L*, kept under the same conditions was so marked that it seems certain that the small race represented a distinct genotype. The size relations of the polyps of the two clones is shown in figure 8, taken from camera lucida sketches of estimated average individuals.

More frequently the differences are less pronounced. Table 15 gives the constants obtained for three clones bred during July and August, 1913. Clones *T* and *L* were continued after *R* was discarded and the constants for their full numbers are given separately. They were grown in individual cultures under parallel conditions as in the first experiment and seem to be hereditarily distinct.

These are the only races which I have cultivated under exactly parallel conditions and no evidence upon the number and variety of such races is available.

*Varieties of Hydra in the earlier literature.* The extensive literature dealing with Hydra gives frequent suggestions of the existence of local varieties of the three generally recognized species, and one or two descriptions seems really to establish their existence. The statements concerning the size of H. viridis made by Baker, Trembley, Rosel, Pallas, and Kästner differ considerably but are not conclusive. Marshall ('82) described a variety of H. viridis from brackish water which remained distinct from the fresh water forms even after cultivation for

many generations in fresh water. The systematic literature and the studies of gonad production by Brauer, Downing Nussbaum, Weltner, and Koelitz, suggest the existence of monoecious and dioecious varieties. The experiments of Tower show two types of H. viridis differing in their reaction to the ultra-violet rays (?) and in the time required for regeneration.[6] Annandale has described a variety of H. grisea from India which is characterized by having a four-tentacled winter form producing gonads. Whitney has been able to establish varieties of H. viridis without green bodies.

*Summary*

To sum up the present section of the work: It has been found that within a wild population of H. viridis there are hereditarily diverse races which differ in their number of tentacles at separation from the parent, in their size at a given age, and less certainly in other characters. The differences between such races are permanent so long as the races are kept under the same environment. The evidence favors the view that the differences are truly genotypic (with the reservation that they are possibly the result of differences in the age of the clones).

## IV. INHERITANCE OF VARIATIONS WITHIN THE CLONE

Inheritance of variations within the clone may be tested in two ways; first, by an analysis of the resemblance between parent and progeny by statistical methods; second, by a continued selection of variates, which should change the type of the clone if the variations are inherited. As has been shown in the discussion of Hanel's data, selection of variates within the clone seems, on the average, to have no effect, yet there is usually a positive correlation of parent and progeny with respect to the selected character. Pearson has subjected Johannsen's data for the selection experiment with beans to a similar analysis and

[6] I have made many attempts to repeat Tower's experiments, using different types of arcs, with and without interposed glass, but have never obtained the sloughing of the ectoderm which he describes.

has shown that in these pure lines there is likewise a correlation between parent and progeny. His explanation of this seemingly anomalous condition and the criticism which has been urged against selection experiments in general where Vilmorin's method is employed is based upon the small numbers selected. Pearson ('10), discussing Hanel's results, says:

> In selecting a few isolated individuals in each generation, where non-hereditary influences are so influential, we may break the influence of heredity at each step, and since such influences are equally effective with heredity, the chances are that we will do so once in every *two* selections. Only by taking large numbers of the high and large numbers of the low would it have been possible to average out the effects of environmental changes.

The only ways in which this criticism can be met are by very extensive selections involving large numbers of individuals, or by a study of variation and environment which shall make possible a differentiation between heritable and non-heritable variations, upon some other basis than that of ancestral correlation.

### *Resemblance of close relatives within the clone*

*Number of tentacles.* In all breeding experiments the polyps have been so recorded that the number of tentacles of the buds can be compared with that borne by their parents, both at the time when the buds were produced and when the parents themselves were buds. Comparisons of both numbers must be made in order to answer two distinct questions: (1) do the offspring resemble their parents when the latter were in the same stage of development: (2) are the variations of the parents during their development transmitted?

To test the first question the correlation between the initial number of tentacles of the parents and that of their buds was computed. Since there is reason to believe that Clone *A* is impure, it was divided into the two subordinate clones mentioned earlier and the constants were computed for these. If there is a cumulative inheritance of slight variations this division should affect only the probable errors of the correlation constants. The

coefficients of correlation obtained in this way are given in table 16. Only one of these, that of Clone $D'$ is more than twice its probable error, and this one is negative. The others are all too small to have any significance. The large negative correlation of Clone $D'$ is the result of the inclusion of a single parent (the stem parent of the clone) which produced a large number of progeny with few tentacles and was thus heavily weighted in the computation of the coefficient.

The Clones $A - (A1A)$ and $D$ contain such large numbers both of parents and offspring that it is almost certain that the low correlation is not the result of chance, but truly expresses an almost total absence of similar variations in parent and progeny within the clone. In so far as the coefficient of correlation is a reliable test of heredity we may conclude that there is no inheritance of variations in the initial number of tentacles of Hydra viridis.

For Clone $D$ the fraternal and grandparental correlations were likewise computed; they are given in table 17. Like the parental correlation, the grandparental is too small to have significance.

TABLE 16

*Correlation of the initial number of tentacles of parents and progeny within the clone*

| | $r$ | NO. OF PARENTS | NO. OF PROGENY |
|---|---|---|---|
| Clone $D$ | $0.0038 \pm 0.018$ | 251 | 1395 |
| Clone $A - (A1A)$ | $0.0011 \pm 0.023$ | | 859 |
| Clone $A1A$ | $0.0342 \pm 0.032$ | | 439 |
| Clone $D'$ | $0.2420 \pm 0.051$ | 18 | 153 |
| Clone $A'$ | $0.0310 \pm 0.047$ | 28 | 204 |
| Clone $T$ | $0.0750 \pm 0.054$ | 51 | 154 |

TABLE 17

*Ancestral correlations, clone D*

| | $r$ | NO. OF PARENTS | NO. OF PROGENY (PAIRS) |
|---|---|---|---|
| Parental | $0.0038 \pm 0.018$ | 251 | 1395 |
| Grandparental | $-0.0495 \pm 0.018$ | 68 | 1307 |
| Fraternal | $0.0770 \pm 0.006$ | | 10766 |

The fraternal is only slightly greater and its value is doubtful. It will be considered again in the discussion of the cause of ancestral correlation within pure lines and clones.

The second question, do the parents transmit to their buds the characters which they have when the buds are formed, involves one of two concepts. Either the effects of environmental action must be transmitted or the organism must continually change in its hereditary potentialities during its development in order that its transient characters shall be inherited. Something of the latter conception seems to be implied in Pearson's doctrine of homotyposis.

TABLE 18

*Correlation between the original number of tentacles of buds and the number borne by the parents at the time when each bud was produced*

| | $r$ | NO. OF PARENTS | NO. OF PROGENY |
|---|---|---|---|
| Clone *D* | 0.096±0.016 | 251 | 1395 |
| Clone *A* | 0.240±0.009 | 242 | 1353 |
| Clone *A*. Grandparental | 0.229±0.013 | | 1094 |

The correlation of the number of tentacles of the buds with that of the number borne by the parents when each bud was produced has been computed for Clones *D* and *A*; these are given in table 18. The high correlation of Clone *A* is obviously the result of the inclusion of the two diverse strains but a comparison of the parental and grandparental correlations within this clone shows that there is a slight positive parental correlation which is not the result of the mixture of the two types. (The parental correlation would not be higher than the grandparental if the inclusion of diverse types were the only cause of correlation). This correlation is certainly very small, and that of Clone *D* is also too small to have significance when considered alone, but the fact that these correlations are greater than the ones obtained for the initial number of tentacles indicates that there is here some factor tending to produce a resemblance between the mature parent and its buds. This is also the impression

given by the average parental correlation of 0.101 found for Hanel's clones.

What is the significance of such a small coefficient of correlation in an organism so subject to environmental influence as is Hydra? Pearson holds that the presence of variations due to environment would tend to obscure the real correlation between parents and offspring and hide any real inheritance which might exist. My data upon the relations of variation to environmental changes indicates that the latter may be more effective in producing a likeness between close relatives than in obscuring such a likeness. Some conditions producing like variations in parent and offspring in Hydra have been considered already. Any great diversities in the conditions of the cultures would reresult in a correlation between parent and progeny, even though there were no inheritance of the variations studied. Such conditions have been found by Agar in daphnids and plant lice and probably account for the ancestral correlations found by Warren in these forms.

The production of a correlation in Hydra by the action of diversities of environment may be illustrated by a consideration of the change in the mean of clones during long periods of cultivation. Figure 5 shows that the mean number of tentacles of the buds produced by Clones *A* and *D* during the first few weeks of cultivation was low; that it increased gradually during the first six weeks, and then decreased again. All the buds produced during each of these five-day intervals were correlated with one another. This gave the following results:

| | *Coefficient of correlation* | *No. of pairs* |
|---|---|---|
| Clone *A* | $0.0774 \pm 0.0015$ | 95141 |
| Clone *D* | $0.1313 \pm 0.0014$ | 101872 |

Correlation of all buds produced in each five day period with all produced in the preceding period (a time interval corresponding to a full generation) gives for Clone *A* a correlation of $0.048 \pm 0.001$ which is greater than any of the parental correlations found for the initial number of tentacles.

These correlations show a resemblance between the buds produced within a limited time as great as that found between the closest relatives, although these buds were no more closely related to each other than to those produced during other five day intervals. This same effect would be visible in the correlation between parent and progeny whenever several generations are included in the same correlation table, as is necessary in the case of Hydra.

Such effects of environmental action seem adequate to account for all the coefficients of correlation given by Hanel's data and for the very slight positive correlations found for the initial number of tentacles in my own clones. The fact that the number of tentacles of successive buds increases with the increase in the number of tentacles of the parent accounts for the slightly larger correlation found between the number of tentacles of the parents and offspring recorded when each bud was produced. Whether the increase in the number of tentacles of the buds is an inheritance of the variations taking place in the parent with growth, or is only a temporary effect of the increased vigor of the parent, must be tested by the success or failure of an attempt to modify the character of the race by the continued selection of variates.

*The effects of selection within the clone*

The second method of testing inheritance, that of continued selection of variates, offers a good many practical difficulties in Hydra owing to the sensitiveness of the polyps to environmental changes. A specimen of H. viridis, collected in the late summer of 1914, was used to found a large clone. Its progeny were bred in individual cultures until 85 members of the clone were obtained. These gave a mean of $6.141 \pm 0.058$ with the distribution shown in table 19. From this clonal population 25 polyps with seven tentacles and 25 with six or less were selected. Each was kept until it produced a bud varying from the mean in the same direction as itself. This bud was then selected and kept in the same way until it in turn produced a bud varying in the same direction, and this selection was continued for three

TABLE 19

*Distribution of variates in the clone from which the fifty parents of the selected lines were taken. The polyp from which this clone was derived bore 9 tentacles*

| Number of tentacles | 3 | 4 | 5 | 6 | 7 | | |
|---|---|---|---|---|---|---|---|
| Number of polyps | 1 | 2 | 9 | 45 | 28 | 85 | |
| Mean | | | | | | 6.141±0.058 | |

months. At the end of this time a record was kept of all the progeny of the last selected generation in each of the 50 lines. The continued freezing of the food pond made it necessary to bring the experiment to a close when an average of 12 buds had been obtained from each of the members of the last selected generation. The selection covered an average of 6.08 generations in the group selected for seven or more tentacles, and of 7.92 generations in the group selected for six or less. The average number of tentacles of all selected generations of the plus selected group was 7.008, that of all selected generations of the minus selected group was 5.560, giving an average of 1.448 tentacles as the amount of difference per generation between the selected ancestors of the two groups.

The first effect of the selection was a marked reduction in the vitality of the group selected for a small number of tentacles. The rate of budding of the group was reduced and some of the polyps showed symptoms of slight depression. Four of the buds of the last selected generation of this group, after maturing and producing from four to eight buds, went into a depression which lasted for a week or more and were revived only with difficulty. The reduction in the vigor of the minus selected group introduces a complication into the study of the effects of selection, for the transmission of reduced vitality, or of characters dependent upon reduced vitality, is not a proof of heredity, unless, indeed, the changed condition prove quite permanent.

The total number of progeny obtained from the last selected generation was 583, of which 309 were from the parents selected for a large number, and 274 from those selected for a small number of tentacles. The distribution of variations in the two

groups is shown in table 20. The offspring of the plus selected group have an average number of tentacles $0.093 \pm 0.035$ higher than that of the minus selected group, which as an effect of selection is scarcely significant. It is about what would be expected for a strength of heredity expressed by the parental correlation of 0.01 (table 21).

Whether this effect of selection proves that the progeny inherit one one-hundredth of the variation of their parents, or is merely the effect of a temporary modification in the vigor of the selected groups may be tested by a comparison of the successive offspring of the last selected generation. Such a comparison shows that the entire difference between the two groups appeared in the first six buds produced. The average number of tentacles of successive buds in the two groups increased

TABLE 20

*Variations in the number of tentacles of the offspring of the last selected generation of polyps selected for a large and a small number of tentacles*

| | 4 | 5 | 6 | 7 | 8 | MEAN |
|---|---|---|---|---|---|---|
| Selected for a large number... | | 7 | 89 | 204 | 9 | $6.695 \pm 0.023$ |
| Selected for a small number... | 2 | 9 | 91 | 165 | 7 | $6.605 \pm 0.026$ |
| Difference in the direction of selection..... | | | | | | $0.095 \pm 0.035$ |

TABLE 21

*Theoretical effect of selection of parents differing by 1.45 tentacles continued for Fn generations when the strength of inheritance is that indicated by the coefficients of correlation given*

| COEFFICIENTS OF CORRELATION | 0.50 | 0.10 | 0.05 | 0.01 |
|---|---|---|---|---|
| Difference at successive generations. | | | | |
| Parental generation..... | 0.000 | 0.000 | 0.000 | 0.000 |
| $F_1$ | 0.725 | 0.145 | 0.073 | 0.015 |
| $F_2$ | 1.098 | 0.276 | 0.141 | 0.029 |
| $F_3$ | 1.274 | 0.393 | 0.207 | 0.043 |
| $F_4$ | 1.362 | 0.498 | 0.269 | 0.057 |
| $F_5$ | 1.406 | 0.594 | 0.328 | 0.071 |
| $F_6$ | 1.428 | 0.689 | 0.384 | 0.084 |

TABLE 22

*Averages of the first six and of all later buds produced by polyps selected for a large and of those selected for a small number of tentacles*

| | AVERAGE OF FIRST SIX BUDS | AVERAGE OF ALL LATER BUDS |
|---|---|---|
| Parents selected for a large number of tentacles | 6.677±0.029 | 6.712±0.030 |
| Parents selected for a small number of tentacles | 6.460±0.034 | 6.782±0.037 |
| Difference in the direction of selection | 0.217±0.044 | −0.070±0.047 |

as usual but those of the minus selected group increased more rapidly and to a greater extent than that of the plus selected one. This is shown in figure 9. The averages of the first six and of all later buds of the two groups is given in table 22. The difference in favor of the plus selected group in the first six buds is

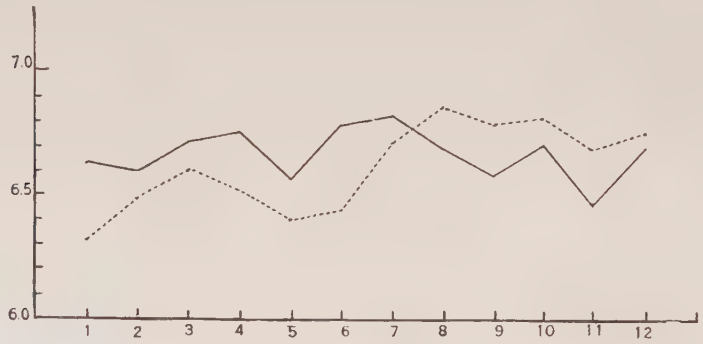


Fig. 9 The average number of tentacles of the successive buds produced by the last selected generation in the selection experiment; (——) ancestry selected for a large number of tentacles; (– – – –) ancestry selected for a small number of tentacles.

0.217±0.044. There is no significant difference between the later buds of the two groups.

Since not all parents produced the same number of buds it seemed possible that this decrease in the difference between the groups might be due to a larger percentage of the progeny of more vigorous polyps among the buds produced after the sixth, but

the data given in figure 9 shows that this is not the case. There is no difference between the averages of the seventh and of the eighth buds of the two groups and all parents produced at least eight buds.

The likeness of this result to that obtained from regenerating polyps (fig. 3) is very striking and there can be little doubt that it is due to the same cause, a reduction in the vitality of one of the groups compared. Selection of polyps with few tentacles resulted in the selection of those with low vitality and as soon as these were given time to regain their health they produced buds varying around the mean of the race. Selection produced no permanent change in the type of the clone studied.

### *Inheritance of size*

From the measurements of the size of polyps in Clones *A* and *D* the correlations in size between parents and offspring and grandparents and grandchildren within these clones were computed. These coefficients are given in table 23. In every case they are positive and the parental correlation of Clone *A* is very high, more than six times that of Clone *D*. The grandparental correlations are negligible but the low grandparental

TABLE 23

*Ancestral correlations for size*

| | COEFFICIENTS OF CORRELATION | COEFFICIENTS OF REGRESSION |
|---|---|---|
| Clone *D* | | |
| Parent and offspring | 0.058±0.035 | 0.056 |
| Grandparent and grandchildren | 0.018±0.036 | 0.020 |
| Clone *A* | | |
| Parent and offspring | 0.358±0.030 | 0.285 |
| Grandparent and grandchildren | 0.030±0.036 | 0.048 |
| Clone *A 1A* | | |
| Parent and offspring | 0.009±0.106 | |
| Clone *A* − (*A1A*) | | |
| Parent and offspring | 0.255±0.063 | |

correlation of Clone *A* shows that the high parental correlation is not wholly the result of the mixture of two races in Clone *A*. Computation of the parental correlation for the two subordinate clones confirms this, giving the following coefficients:

| | *Coefficient of correlation* |
|---|---|
| Clone *A1A* | 0.009±0.106 |
| Clone *A* − (*A1A*) | 0.255±0.063 |

After this division the correlation is still significant and is four times as great as that found for Clone *D*. An examination of the data for possible environmental causes of correlation

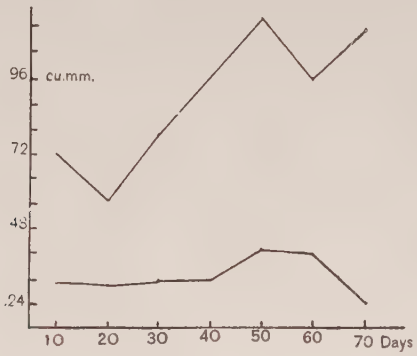


Fig. 10 Changes in the average size of the buds produced during successive ten-day intervals by Clones *A* and *D*. The ordinates represent the mean sizes in cubic millimeters of all measured buds produced during each ten-day period; the abscissae, the successive ten-day periods.

gives the result shown in figure 10. As in the case of the number of tentacles, there was a considerable change in the mean size of the buds produced at different times during the experiment.

The correlations between all buds produced in each of the five-day intervals were:

| | *Coefficient of correlation* |
|---|---|
| Clone *D* | 0.0118 |
| Clone *A* − (*A1A*) | 0.0952 |

There is here the same difference in the size of the correlations of the two clones which appeared in the parental correlation but

the correlation due to the changing clonal mean is too small to account for the parental correlation. No adequate data for a further analysis of the correlation in size is at hand, and it may be that variation in size within the clone is actually inherited but, although conclusive evidence against this is lacking, too much trust should not be placed in the parental correlations.

The following points, while based only upon general impressions gained during the experiments and subject to correction by further experimental test, indicate some of the factors which may have been instrumental in producing a high parental correlation for size. Size is a character which is modified much more readily and quickly than the number of tentacles by changes in the environment. Hydras, placed in a 0.1 per cent solution of NaCl, in a very few days become dark in color, small, and produce small, dark-colored buds. Similar changes in size follow extreme changes in temperature. Upon the restoration of optimum conditions the normal size is resumed very quickly. Starvation is effective in much the same way but to a lesser extent. When a Hydra has been injured slightly, or has passed through a slight depression it grows smaller and produces small buds but eventually both buds and parents resume the normal size of the clone. The size, indeed, seems to be a matter of the immediate state of nutrition of the polyps. Clone *A* was more readily modified by such agents than was Clone *D*. This is shown by the following data for the number of tentacles of polyps from mass cultures with and without food for three months:

| *3-month mass cultures* | *With food* | *Without food* | *Difference* |
|---|---|---|---|
| Clone *A* | 6.80±0.06 | 5.52±0.04 | 1.28±0.07 |
| Clone *D* | 5.52±0.05 | 5.34±0.03 | 0.18±0.06 |

and by the changes represented in figure 10. Corresponding to this fact, the correlations between the relatives in Clone *A* are higher than in Clone *D*, which justifies the suspicion that the correlation between parent and offspring for size within the clone is really due only to the action of the environment.

## V. DISCUSSION OF RESULTS

The experiments reported show that populations of Hydra viridis consist of races which have different hereditary constitutions. The diversity between two such strains persisted for so long as they were kept under observation (143 days) and so long as the different strains were kept under similar and favorable conditions they showed no tendency to approach each other in character. The distinguishing characters of the different races studied were not, however, fixed in the sense of remaining constant through fluctuations in the environment but underwent changes corresponding to changes in the environment. In general, the diverse clones responded to such changes in the same way and to the same degree, although in the face of very unfavorable conditions the larger strain was most affected and under conditions of almost complete starvation the strains became much more similar.

Little evidence has been obtained as to the cause and fundamental nature of the difference between the clones. The diverse characters noted, number of tentacles, size, color, and age at which asexual reproduction is begun, may all be modified by changing the environmental conditions and the changes thus produced in the first three characters mentioned are correlated in the same way that they are in the diverse races (large size, many tentacles, and light color occur together) so that it is possible that the diversities in these three characters are due to a difference in some single set of physiological processes. Failure to obtain sexual reproduction in H. viridis has made it impossible to determine the relation of the diverse races to gametic processes and to the supposed life cycle of Hydra but there is certainly no relation between the diversity of the clones and the phenomena of 'depression' which have been thought to mark periods in the life cycle.

The existence of diverse races of Hydra is in accord with the results of Jennings, Woltereck, Shull, Whitney and Agar gained from the study of clones of other invertebrates and, indeed, the volume of evidence from zoological and botanical literature

leaves no doubt that the existence of diverse races within the species is a general condition in all phyla.

The problem of inheritance of variations within the clone presents much greater difficulties and there is much conflict between the results of different investigators. The work of Whitney has shown that in Hydatina diverse strains may arise in a clone descended from a single fertilized egg and Calkins and Gregory report similar results for Paramecium. In these cases there is, however, no intimation that the inheritance of variations is a general characteristic of asexual reproduction or that the change is the result of the accumulation of slight variations. The four studies in which there is an appearance of inheritance of continuous variations within the pure line or clone are those summarized by Pearson in 1910; the studies of Johannsen on beans, Warren on Daphnia and Hyalopterus, and Hanel on Hydra. In all this work the evidence for inheritance can be drawn only from the ancestral correlations, while the evidence from the effects of selection seems to point the other way. The question of the relative values of the coefficient of correlation and of selection experiments hence becomes of great importance.

In Agar's recent study of inheritance in parthenogenesis, where great precautions were taken to rule out the influence of environmental agents, there is no significant correlation between parent and progeny within clones of Cladocera and sufficient evidence of environmental causes of correlation is presented to account for Warren's results with Daphnia. For Aphids he finds, with Warren, a slight ancestral correlation but the evidence for an environmental cause of this correlation, while perhaps not absolutely conclusive, is sufficient to make the ancestral correlation in these forms of very doubtful value as an index of inheritance. Ewing's selection experiments with Aphis avenae offer further evidence against the inheritance of variations in these forms.

The results recorded in the present paper are quite in accord with those of Agar on Cladocera and show that for Hydra also the ancestral correlation is untrustworthy as a measure of inheritance.

There remain only Johannsen's data on Phaseolus giving evidence of an ancestral correlation within the pure line. The evidence adduced by Pearson indicates only a slight correlation here and the recent work of Harris ('12) upon the transmission of the effects of unfavorable cultural conditions indicates that the real cause of this correlation is likewise environmental.

## VI. SUMMARY

1. Populations of Hydra consist of hereditarily distinct strains which differ in initial number of tentacles, size of body, color, age at which asexual reproduction is begun, and perhaps in other characters.

2. In the absence of selection these strains remain distinct.

3. Within populations there is a correlation between the characteristics of parents and progeny and of other close relatives, which is largely due to the existence of these diverse strains.

4. Within the clone there is no significant correlation between the variations of close relatives in the initial number of tentacles.

5. Within the clone there is a slight correlation between the number of tentacles of the buds and the number of tentacles which their parents bear when each bud is produced.

6. Diversities of environment tend to produce like variations in parents and offspring and this likeness tends to disappear when the environmental cause is removed. The existence of such environmental agents is sufficient to account for the ancestral correlations found, even though there is no inheritance of variations.

7. Continued selection of variates in tentacle number results in changes in the vigor of the selected groups. This results in an apparent diversity of the differentially selected groups but the diversity persists only during selection and disappears at once when selection is discontinued. Variations in the number of tentacles of Hydra viridis are not inherited.

8. There is a positive correlation between the variations in the size of parent and offspring within the clone. No statistical evidence of an external cause of this correlation is presented

but from general considerations it seems probable that this, like the correlation of variations in the number of tentacles, is due wholly to the similar action of environmental agents upon parent and offspring.

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## APPENDIX

The correlation tables from which the principal constants given in the body of the paper were obtained are appended here. In all cases where the tables include an earlier and a later generation the ascendants are arranged in the rows, the descendants in the columns. In those cases where there is no qualitative difference between the members of pairs, as when siblings are correlated, the pairs are entered only once, according to the method recommended by Jennings ('11). The tables from which the correlations for size were computed are not included because of their great bulk and the lack of certain evidence upon the environmental modification of size.

TABLE 24

*Clone D: Correlation between the initial numbers of tentacles of parents and offspring*

| | 4 | 5 | 6 | 7 | 8 | |
|---|---|---|---|---|---|---|
| 4 | | 12 | 17 | 1 | | 30 |
| 5 | 23 | 126 | 233 | 7 | 5 | 394 |
| 6 | 76 | 193 | 542 | 49 | 23 | 883 |
| 7 | 6 | 37 | 41 | 2 | | 86 |
| 8 | 1 | 1 | | | | 2 |
| | 106 | 369 | 833 | 59 | 28 | 1395 |

$r = 0.0038 \pm 0.018$

TABLE 25

*Clone D: Correlation between the initial numbers of tentacles of grandparents and grandchildren*

| | 4 | 5 | 6 | 7 | 8 | |
|---|---|---|---|---|---|---|
| 4 | 2 | 9 | 27 | | | 38 |
| 5 | 16 | 110 | 242 | 7 | | 375 |
| 6 | 84 | 211 | 505 | 11 | 5 | 816 |
| 7 | 11 | 13 | 51 | 1 | | 76 |
| 8 | | 1 | 1 | | | 2 |
| | 113 | 344 | 826 | 19 | 5 | 1307 |

$r = -0.0495 \pm 0.018$

TABLE 26

*Clone D: Correlation between the numbers of tentacles of members of the same fraternity*

| | 4 | 5 | 6 | 7 | 8 | |
|---|---|---|---|---|---|---|
| 4 | 6 | 133 | 273 | 24 | | 436 |
| 5 | | 896 | 3503 | 290 | 12 | 4701 |
| 6 | | | 4550 | 969 | 31 | 5550 |
| 7 | | | | 77 | 2 | 79 |
| | 6 | 1029 | 8326 | 1360 | 45 | 10766 |

$r = 0.077 \pm 0.006$

TABLE 27

*Clone D: Correlation of the initial number of tentacles of the offspring with the number of tentacles borne by the parents when each bud was produced.*

| | 4 | 5 | 6 | 7 | 8 | |
|---|---|---|---|---|---|---|
| 4 | | 9 | 19 | 2 | | 30 |
| 5 | 6 | 95 | 267 | 21 | 5 | 394 |
| 6 | 8 | 119 | 623 | 110 | 23 | 883 |
| 7 | | 32 | 45 | 9 | | 86 |
| 8 | | | 1 | 1 | | 2 |
| | 14 | 255 | 955 | 143 | 28 | 1395 |

$r = 0.096 \pm 0.016$

TABLE 28

*Clone A-(A1A): Correlation between the initial number of tentacles of parent and offspring.*

| | 5 | 6 | 7 | 8 | 9 | |
|---|---|---|---|---|---|---|
| 4 | | | 3 | | | 3 |
| 5 | 1 | 18 | 17 | 6 | | 42 |
| 6 | 14 | 140 | 109 | 31 | 2 | 296 |
| 7 | 23 | 222 | 133 | 67 | 10 | 455 |
| 8 | 4 | 31 | 13 | 13 | 1 | 62 |
| 9 | | 1 | | | | 1 |
| | 42 | 412 | 275 | 117 | 13 | 859 |

$r = 0.0011 \pm 0.023$

TABLE 29

*Clone A1A: Correlation between the initial number of tentacles of parent and offspring*

| | 4 | 5 | 6 | 7 | 8 | |
|---|---|---|---|---|---|---|
| 4 | | | 3 | 1 | | 4 |
| 5 | 1 | 9 | 32 | 8 | | 50 |
| 6 | 4 | 61 | 177 | 32 | 4 | 278 |
| 7 | | 31 | 62 | 9 | 2 | 104 |
| 8 | | | 2 | 1 | | 3 |
| | 5 | 101 | 276 | 51 | 6 | 439 |

$r = -0.0342 \pm 0.032$

TABLE 30

*Clone A: Correlation of the initial number of tentacles of the offspring with the number of tentacles borne by the parents when each bud was produced*

| | 5 | 6 | 7 | 8 | 9 | 10 | |
|---|---|---|---|---|---|---|---|
| 4 | | 3 | 5 | | 1 | | 9 |
| 5 | 7 | 40 | 32 | 11 | 3 | | 93 |
| 6 | 24 | 255 | 182 | 101 | 23 | 5 | 590 |
| 7 | 16 | 125 | 221 | 159 | 63 | 5 | 589 |
| 8 | 3 | 9 | 28 | 21 | 8 | 9 | 69 |
| 9 | | | | 1 | 2 | | 3 |
| | 50 | 432 | 468 | 293 | 100 | 10 | 1353 |

$r = 0.240 \pm 0.009$

TABLE 31

*Clone A: Correlation between the initial number of tentacles of grandparents and grandchildren*

| | 5 | 6 | 7 | 8 | 9 | |
|---|---|---|---|---|---|---|
| 4 | 2 | 1 | 1 | 2 | | 6 |
| 5 | 4 | 24 | 15 | 15 | 3 | 61 |
| 6 | 39 | 139 | 139 | 104 | 12 | 433 |
| 7 | 36 | 53 | 155 | 219 | 47 | 510 |
| 8 | 8 | 7 | 18 | 48 | | 81 |
| 9 | | | 2 | 1 | | 3 |
| | 89 | 224 | 330 | 389 | 62 | 1094 |

$r = 0.229 \pm 0.019$

TABLE 32

*Clone D'. Correlation between the initial numbers of tentacles of parents and offspring*

| | 4 | 5 | 6 | 7 | |
|---|---|---|---|---|---|
| 4 | | | 1 | 1 | 2 |
| 5 | 1 | 2 | 14 | 12 | 29 |
| 6 | 3 | 7 | 67 | 37 | 114 |
| 7 | 3 | | 3 | 1 | 7 |
| 8 | 1 | | | | 1 |
| | 8 | 8 | 85 | 51 | 153 |

$r = -0.242 \pm 0.051$

TABLE 33

*Clone A'. Correlation between the initial numbers of tentacles of parents and offspring*

| | 6 | 7 | 8 | 9 | |
|---|---|---|---|---|---|
| 5 | 1 | 2 | | | 3 |
| 6 | 3 | 14 | 11 | 5 | 33 |
| 7 | 24 | 78 | 34 | 12 | 148 |
| 8 | | 10 | 7 | 3 | 20 |
| | 28 | 104 | 52 | 20 | 204 |

$r = 0.031 \pm 0.047$

TABLE 34

*Clone A: Correlation between the numbers of tentacles of all buds produced in each arbitrary five-day period. Each bud is paired in the table with all the others produced in the same period*

| | 4 | 5 | 6 | 7 | 8 | 9 | |
|---|---|---|---|---|---|---|---|
| 4 | 2 | 82 | 355 | 270 | 20 | | 729 |
| 5 | | 574 | 5572 | 4300 | 484 | 4 | 10934 |
| 6 | | | 17277 | 35967 | 3859 | 102 | 57205 |
| 7 | | | | 20742 | 4982 | 254 | 25978 |
| 8 | | | | | 264 | 30 | 294 |
| 9 | | | | | | 1 | 1 |
| | 2 | 656 | 23204 | 61279 | 9609 | 391 | 95141 |

$r = 0.0774 \pm 0.0015$

TABLE 35

*Clone A: Correlation of the numbers of tentacles of all buds produced in each five-day period with all buds produced in the preceding five-day period. Each bud is paired individually with all buds produced in the preceding period*

| | 4 | 5 | 6 | 7 | 8 | 9 | |
|---|---|---|---|---|---|---|---|
| 4 | 9 | 87 | 374 | 372 | 42 | 1 | 885 |
| 5 | 66 | 846 | 4999 | 4552 | 461 | 5 | 10929 |
| 6 | 245 | 5084 | 32259 | 33039 | 3897 | 81 | 74605 |
| 7 | 153 | 3973 | 32873 | 38143 | 4650 | 94 | 79886 |
| 8 | 16 | 495 | 3866 | 4352 | 527 | 9 | 9265 |
| 9 | | 4 | 55 | 93 | 11 | | 163 |
| | 489 | 10489 | 74426 | 80551 | 9588 | 190 | 175733 |

$r = 0.048 \pm 0.001$

TABLE 36

*Clone D: Correlation between the numbers of tentacles of all buds produced in each arbitrary five-day period; arranged as in table 34*

| | 4 | 5 | 6 | 7 | 8 | |
|---|---|---|---|---|---|---|
| 4 | 91 | 1845 | 2936 | 261 | 12 | 5100 |
| 5 | | 8993 | 33381 | 1993 | 72 | 44439 |
| 6 | | | 41613 | 9265 | 484 | 51362 |
| 7 | | | | 797 | 168 | 965 |
| 8 | | | | | 6 | 6 |
| | 91 | 10838 | 77930 | 12271 | 742 | 101872 |

$r = 0.1313 \pm 0.0014$

## VITA

I was born at Davis, W. Va., on June 7, 1890, the son of C. G. Lashley, a retail merchant, and Blanche M. Lashley, *nee* Spencer. I attended public schools in West Virginia and Washington until 1905 when I entered the West Virginia State Preparatory School. The following year I entered the State University. From 1908 to 1910 I was student assistant to Professor A. M. Reese, to whom I owe the first direction of my interests to biological work. I received the degree of Bachelor of Arts in 1910 and during the following year held the position of 'Teaching Fellow in Biology' at the University of Pittsburgh. There I worked in bacteriology with Professor J. C. Fettermann, receiving the degree of Master of Science in 1911. Since that time I have been in attendance at the Johns Hopkins University, studying chiefly with Professor H. S. Jennings in zoölogy, Professor J. B. Watson in psychology, and Professor Adolph Meyer is psycho-pathology. In 1913 I was appointed University Fellow in the Department of Zoölogy of the Johns Hopkins University.

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